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Conchotome and needle percutaneous biopsy of skeletal muscle

P DIETRICHSON,* J COAKLEY, P E M SMITH, R D GRIFFITHS, T R HELLIWELL, R H T EDWARDS

From the Muscle Research Centre, University Department of Medicine, Royal Liverpool Hospital, Liverpool, UK

SUMMARY Percutaneous muscle biopsy is an important and acceptable technique in the study of conditions involving human skeletal muscle. A review of 436 conchotome and needle muscle biopsies obtained over 18 months in this centre is presented.

Muscle biopsy is an important tool in the investigation of diseases of muscle, nervous system and connective tissues. The percutaneous techniques have clear advantages over open muscle biopsy, especially regarding the patient's comfort and the absence of unsightly scarring. Despite the small incision, however, specimens can be obtained of suitable size and quality to meet the majority of needs. There is now a large experience of needle muscle biopsy (fig 1a) in this country.[1 2] It is recognised as a simple, rapid and repeatable method of obtaining muscle tissue, safely performed on an outpatient, and applicable to patients of all ages.[3] We have recently adopted the technique of percutaneous biopsy using a conchotome[4] (fig 1b), an instrument primarily intended for nasal surgery, and already in use for muscle biopsy in Scandinavia.[5–8] The conchotome adds further flexibility to investigation of muscle pathology, enabling biopsies to be taken from small muscles unsuitable for needle sampling. Both techniques are in routine use in our unit.

In this article we outline our recent experience of percutaneous muscle biopsy, with particular reference to conchotome biopsy of the anterior tibial muscle, and we reinforce the case for percutaneous sampling over open muscle biopsy.

Patients

The age range of the 292 subjects (including 65 children aged 16 years or under) biopsied during the past 18 months was 8 days to 70 years. The diagnostic groups studied are shown in table 1; most of the groups consisted of single diagnoses, while others require further explanation.

The 26 patients in the miscellaneous group included two with rhabdomyolysis, two with malignant hyperpyrexia, three with benign myokymia, three with connective tissue disorders and others with a variety of disorders such as muscle wasting occurring in the intensive care unit, toxoplasma myositis and raised serum creatine kinase levels without obvious clinical evidence of muscle disease.

Sixty two patients are grouped under the heading of "effort syndromes". These patients complained of muscle pain on or after exercise and variable degrees of fatigue or weakness; their symptoms frequently dated from the time of an acute viral-like illness. Specimens of muscle from these patients were examined by electron microscopy and tissue innoculation (in addition to routine light microscopy and mitochondrial studies) in an attempt to obtain evidence of continuing viral infection. Specimens of muscle from three patients with defined muscle diseases and eight healthy controls were also submitted to virological examination.

Some patients presenting with pain and weakness on effort ultimately fell into other diagnostic groups (for example endocrine myopathies, metabolic myopathies) and these are included under the appropriate headings. Most of the latter had clinical signs or laboratory evidence of organic muscle disease in contradistinction to the "effort syndrome" patients, who generally demonstrated no physical signs or abnormal laboratory investigations.

The miscellaneous neurological group includes patients with hereditary sensorimotor neuropathies, chronic inflammatory demyelinating polyneuropathies, and Friedreich's ataxia.

The metabolic myopathies studied were McArdles disease, acid maltase deficiency, and carnitine palmitoyl transferase deficiency.

The control subjects were biopsied either as part of a clinical trial of selenium metabolism or as part of a study of the normal structure and function of the anterior tibial muscle: the approval of the Ethical Committee of the Royal Liverpool Hospital was obtained for these studies, and for those

Present address: Ullevaal Hospital, Neurological Department, 0407, Oslo 4, Norway.
Address for reprint requests: Professor R H T Edwards.

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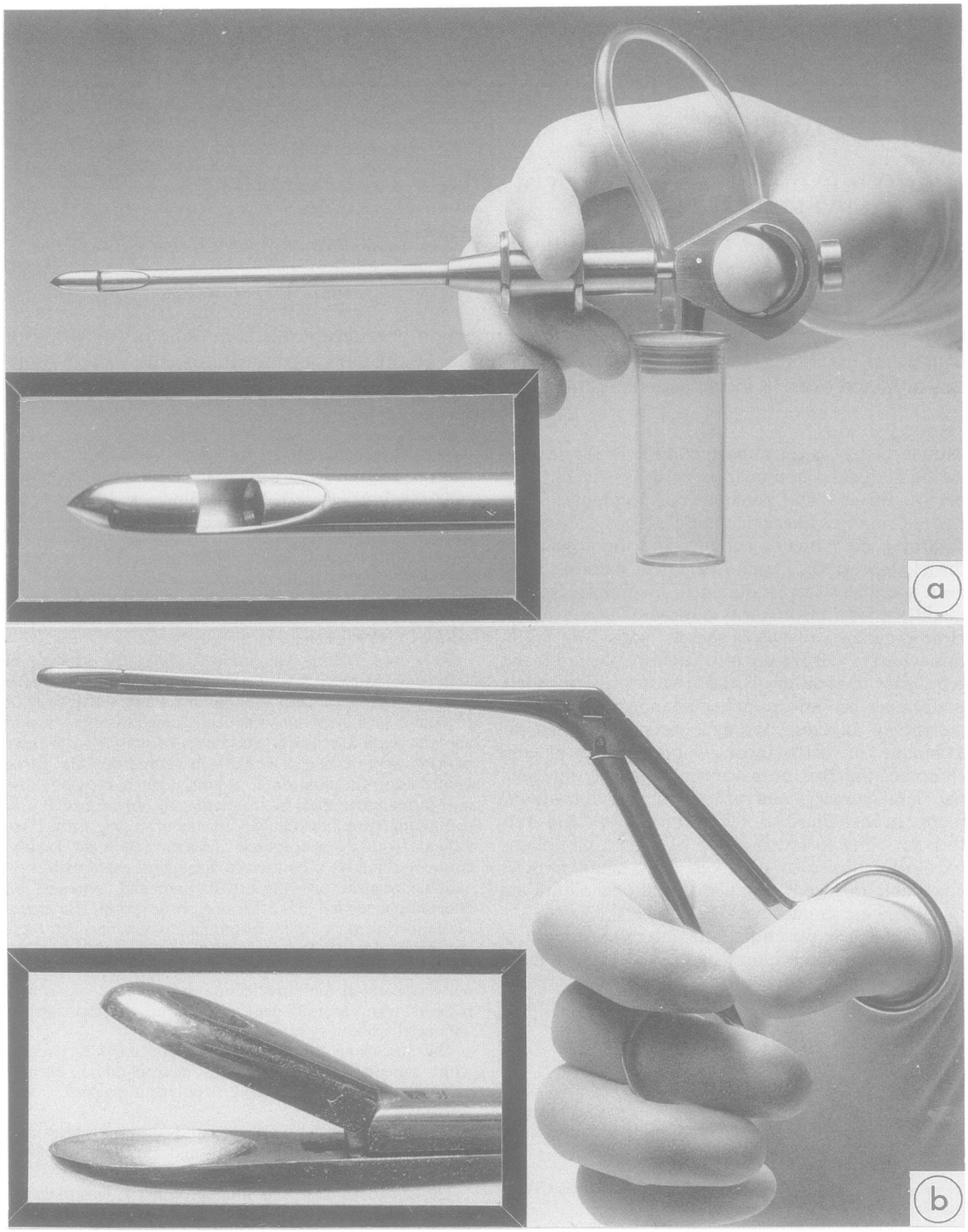

Fig 1(a) *Liverpool muscle biopsy needle.* (b) *Conchotome biopsy forceps.*

Table 1 *Diagnostic groups studied, destination of biopsies and percentage with histological abnormalities*

Diagnosis	*Age mean (range) Years*	*Number of Patients*	*Number of male patients*	*Number of biopsy occasions*	*Number of biopsies*	*Conchotome*	*Needle*	*Samples for histology*	*% Abnormal histology*	*Samples for mitochondrial study*	*Samples for trial*	*Samples for biochem*	*Samples for viral study*
Duchenne MD	9·5 (8 days–18 yrs)	26	26	31	31	10	21	17	100	9	14	10	0
Becker MD	22·4 (13–38)	13	13	17	20	10	10	12	100	1	9	1	0
Limb Girdle MD	39·3 (3–66)	33	18	41	48	26	22	32	100	6	19	1	0
FSH MD	37·1 (12–56)	31	15	36	41	18	23	27	85	2	18	0	0
Myotonic Syndromes	36·8 (17–56)	13	10	15	18	8	10	14	79	0	6	0	0
Inflammatory myopathy	47·1 (3–70)	13	5	19	28	8	20	27	100	3	0	5	1
Endocrine myopathy	43·6 (28–65)	8	5	8	10	3	7	10	70	2	0	1	0
Metabolic myopathy	35·2 (17–50)	17	6	24	34	25	9	25	84	21	0	1	2
Effort syndrome	36·3 (14–65)	62	24	67	83	55	28	79	3	28	0	5	10
Spinal muscular atrophy	15·6 (14 days–47 yrs)	9	5	9	11	4	7	11	100	0	0	0	0
Miscellaneous Neurological conditions	25·5 (4–53)	15	11	15	21	12	9	19	79	3	0	0	0
Mitochondrial myopathy	29·0 (10 wks–65 yrs)	5	3	5	5	3	2	5	100	4	0	0	0
Congenital myopathy	12·7 (1–21)	6	3	7	8	2	6	7	100	0	0	0	0
Control Subjects	32·1 (24–48)	15	14	33	40	20	20	9	0	24	30	1	8
Miscellaneous	35·5 (1·5–61)	26	16	30	38	18	20	40	45	12	0	4	0
TOTAL	32·4 (8 days–70 yrs)	292	174	357	436	222	214	330	63	115	96	29	21

trials where patients with muscular dystrophy were studied. The selenium study is not yet complete but preliminary results have been published in abstract form.[9]

Selection of the muscle

Where possible, a muscle was chosen which was only moderately affected. Severely atrophic muscles are unsuitable (see below) because end stage histological features of both neuropathic and myopathic disorders can be similar. In suspected myositis or vasculitis a tender area may be the most suitable for biopsy and most likely to reveal diagnostic abnormalities. Where involvement is patchy more than one muscle may be sampled. Techniques such as CT scanning,[10] ultrasound scanning[11] and radionuclide imaging[12] can all identify areas of abnormal muscle in difficult cases.

The anterior tibial muscle (with the conchotome) and quadriceps or gastrocnemius (with the needle or conchotome) are the muscles with which we have the most experience in adults, but paraspinal, triceps and deltoid muscles have been sampled by the needle method at other times, and we have used the conchotome technique to biopsy biceps, deltoid, trapezius and supraspinatus muscles (table 2).

Table 2 *Site of biopsy with conchotome and needle*

Site	*Conchotome*	*Needle*
Vastus lateralis	15	112
Gastrocnemuis	18	102
Anterior tibial	178	0
Trapezius	4	0
Deltoid	3	0
Biceps brachii	2	0
Erector spinae	1	0
Supraspinatus	1	0
TOTAL	222	214

Methods of percutaneous biopsy

All our muscle biopsies were performed with local anaesthesia; no general anaesthetic was ever required. Exceptionally, in very anxious patients, 5–10 mg of diazepam was administered intravenously shortly before the procedure. We have found that even young children tolerate muscle biopsy well without sedation, and where appropriate a parent may remain with the child throughout the procedure to provide additional reassurance. Biopsies were usually performed in a side room set aside for this purpose, but may be performed in the ward, out-patients department, intensive care unit, or in the laboratory. A nurse or technician assisted the physician performing the procedure. The technique was little more troublesome than blood sampling. A clotting screen and platelet count was obtained before biopsy if there was a history of easy bruising or bleeding, but was not necessary in the absence of such a history.

(a) Conchotome technique

Routine aseptic precautions were taken. The skin was shaved, and cleaned with chlorhexidine in alcohol or other suitable disinfectant. The biopsy site was infiltrated with 5 ml of 2% lignocaine taking care to inject skin and subcutaneous tissue, but avoiding the muscle itself. With the tibialis anterior it was possible in addition to perform a local nerve block by the injection of lignocaine just below the head of the fibula. It was also possible to anaesthetise any muscle by direct infiltration proximal to the biopsy site. We have found, however, that adequate analgesia was usually achieved from local infiltration alone. A 5 mm skin incision was made, and the fascia penetrated with a scalpel blade. The closed jaws of the conchotome were inserted through the incision and into the muscle with the long axis of the jaws parallel to the muscle fibres. The jaws were opened and the conchotome advanced 2–3 mm. In a single movement the

jaws were closed, twisted through 180° and withdrawn. A scalpel blade or sterile needle was used to deliver the sample on to a gauze swab. The procedure could be repeated several times through the same incision. Each biopsy weighed 50–150 mg. Pressure was applied to the biopsy site for 5–10 minutes to stop bleeding and prevent haematoma formation. Adhesive strips were used to close the incision. The patient was asked to rest for 30 minutes after the procedure, but might then walk, though strenuous exercise is best avoided for 24 hours. It was not necessary to sharpen the jaws of the conchotome as the cutting ability was well maintained for up to 4 years. The twisting motion was not always necessary, but ensured that all the muscle fibres contained within the conchotome were severed before the conchotome was withdrawn. This was particularly important when the muscle was tough and fibrous, as in muscular dystrophy. In general we recommend the use of the smaller conchotomes in younger patients (for example 4·5 mm size) and the bigger conchotome in larger patients (for example 6·5 mm size).

(b) Needle biopsy technique

We have recently, in conjunction with the manufacturers, developed a modified needle (fig 1a), which has an adjustable thumb grip to maintain low grade suction (approximately -10 kPa (-100 cm H_2O)) throughout the procedure. This needle produces samples of 100 mg or more. The suction enables up to three samples to be taken during a single insertion either from different positions by rotating the needle, or at different depths by inserting the needle further. Again asepsis is the rule, and the technique for local anaesthesia is the same as described for the conchotome. The needle is passed through the skin incision and through the fascia, with the aperture in the closed position. When the muscle is entered, the aperture is opened by withdrawing the inner tube or "guillotine" and then closed trapping a sample of muscle in the barrel. As noted above, up to three samples are obtained with a single penetration. Again pressure is applied for 10 minutes and adhesive strips used to close the incision. Great care must be taken to ensure that the cutting edge of the guillotine is not damaged, and it should be sharpened regularly.

Specimen processing

The techniques for processing muscle specimens have been well described.[1 3] For most histology and histochemistry it is acceptable to transport the specimen to the laboratory in a plastic specimen bottle, even if this means a 20 minute delay in processing. Specimens for histology are inspected on a cork block under a dissecting microscope, and orientated so that muscle fibres are perpendicular to the block. This confirms the presence of muscle tissue and ensures that true cross sections are obtained. The specimens are then frozen in isopropanol cooled in liquid nitrogen. The specimens are routinely stained with haematoxylin and eosin, periodic acid Schiff, oil red O, modified Gomori trichrome, NADH-tetrazolium reductase (NADH-TR), succinic dehydrogenase, phosphofructokinase, myophosphorylase, and adenylate deaminase. Staining for myosin ATPase activity is carried out at pH 9·5, 4·6, and 4·3. For biochemical studies samples are placed in a plastic specimen tube, and rapidly frozen in liquid nitrogen, although studies of intact mitochondria require fresh unfrozen muscle.[13 14]

Results

In the past 18 months we have taken 436 percutaneous muscle biopsies on 357 occasions, from a total of 292 subjects (60% male). In 79 subjects, biopsies were taken from two sites on one occasion, and 44 subjects had biopsies taken on 2–5 occasions during the period of study. The conchotome was used for 222 biopsies, and the needle for 214. The muscles sampled with each technique are shown in table 2. In all cases material adequate for diagnostic purposes was obtained.

Histological examination was performed on 330 muscle specimens and abnormalities were seen in 208. The percentage of biopsies showing abnormal histological appearances in each of the diagnostic categories is shown in table 1.

In most groups there was a high proportion of diagnostic abnormalities. In other groups, particularly those where muscle involvement is patchy, such as facioscapulohumeral dystrophy, or where muscle involvement is not invariable, such as endocrine myopathies and some neurological conditions, the percentage of histological abnormalities was slightly less. In the "effort syndrome" patients there was a negligible proportion of histological abnormalities; two of the 62 patients had non-specific abnormalities suggestive in one case of a neuropathic process, and in the other of a myopathic process. The former patient

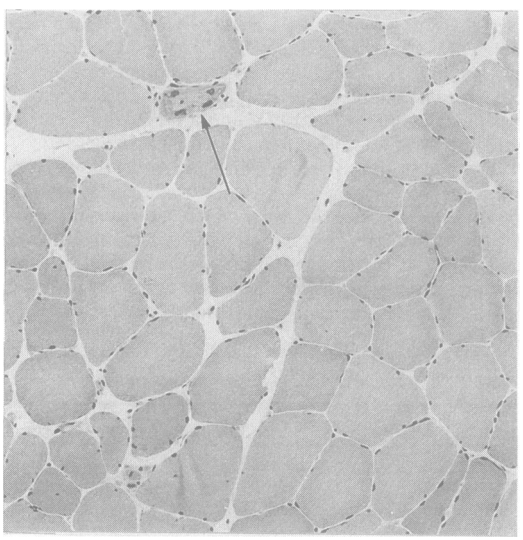

Fig 2 *Facioscapulohumeral dystrophy: deltoid muscle showing mild myopathic changes including atrophic and hypertrophic fibres, endomysial fibrosis and a single regenerating fibre (arrow) (H and E × 130).*

Table 3 *Mean fibre area of normal anterior tibial muscle*

		Type I fibres	*Type II fibres*
Males	Mean area ± SD (μm^2)	4661 ± 791	7286 ± 1116
Females	Mean area ± SD (μm^2)	3372 ± 947	4590 ± 840

made a complete clinical recovery 6 months after his initial presentation, and muscle biopsy was not repeated. The latter patient had a normal muscle biopsy taken previously from the gastrocnemius, the abnormal sample coming from the supraspinatus. Although he had initially complained of generalised muscle pain, it later transpired that his job as a fitter imposed major strains on his shoulder girdle musculature, and it was here that his symptoms were most prominent. His symptoms have eased in the year since he came under our care without any specific treatment. The precise explanation for the minor abnormalities in his muscle biopsy is not clear.

None of the samples sent for virological study showed evidence of persistence of virus particles in healthy subjects or patients.

The number of samples submitted to biochemical analysis or detailed study of mitochondrial function is shown in table 1, but a detailed discussion of these results is outside the scope of this article.

Twenty seven biopsies from the anterior tibial muscle were examined histologically and a detailed description of morphometry and morphological data published elsewhere.[15]

Briefly, the percentage of Type I fibres ranges from 70–85% in males and 65–90% in females. The mean values for fibre area are shown in table 3, and are derived from seven healthy male volunteers, and 10 females with "effort syndrome". "Moth eaten" fibres are seen in nearly all anterior tibial biopsies and are considered by us to be a normal finding in this muscle.

In the experience of all the healthy volunteers who had submitted themselves to both conchotome and needle biopsy techniques, the conchotome was considerably less painful. In the vast majority of patients, many of whom have also experienced both types of biopsy, the conchotome was preferred.

Discussion

Muscle composes 40% of the body mass, and often reflects disorders in other systems. The study of muscle morphology and chemistry is, therefore, of interest to physicians other than the neurologists, paediatricians and rheumatologists who have traditionally dealt with muscle disease.[16-19] Thus exercise physiologists have studied exercise induced changes in glycogen and electrolytes in biopsy samples[16] and gastroenterologists have studied changes in morphology and electrolyte content during dietary manipulations.[17] In addition muscle biopsy studies have been made in postoperative patients[18] and uraemic patients.[19]

Percutaneous muscle biopsy by conchotome or needle is preferable to open biopsy for most situations. Needle biopsy, originally described by Duchenne[20] and modified by Bergstrom[21] and Edwards,[22] has become widely accepted over the past decade.[3 23] The conchotome biopsy method, although employed in Scandinavia for 20 years[5 8] has, until now, not been used routinely in the UK. Specimens obtained by either method are satisfactory for most histological and histochemical purposes, and may also be used for detailed biochemical and mitochondrial function studies.[13 14]

A major advantage of the conchotome technique is that the range of muscles available for percutaneous biopsy has been broadened considerably. This is especially important in conditions where there is selective muscle involvement (such as facioscapulohumeral dystrophy) or severe degeneration of the large proximal groups (such as advanced limb girdle syndrome). The technique has enabled us to make a positive diagnosis of limb girdle dystrophy rather than a neuropathic process in a patient with very wasted lower limb musculature from whom it proved impossible to take a diagnostically useful biopsy from the proximal leg mucles because of fibrous and fatty replacement, or distal leg muscles because of gross oedema. In addition, there was marked wasting of the arm muscles. Using the conchotome, a biopsy of the trapezius muscle was taken, and a definitive diagnosis made. A biopsy taken from the deltoid muscle (fig 2) of a patient with facioscapulohumeral dystrophy showed changes compatible with a dystrophic process. The needle has been used previously to sample shoulder girdle muscles, but this requires deep penetration into the muscle before sampling; a more superficial and safer biopsy can be taken with the conchotome.

Most experience with the conchotome technique has been with the anterior tibial muscle.[7 8] Our experience suggests that this is a more comfortable site for muscle biopsy than the quadriceps or calf; a local nerve block can, in certain cases, provide additional anaesthesia, though we do not find it desirable or necessary in most cases.

The histological findings in the anterior tibial muscles of healthy male volunteers and in patients with "effort syndromes" have been previously reported[15] and are similar to those described in necropsy reports of previously healthy males and females who suffered sudden accidental death.[24-27] Interpretation of pathological changes in the anterior tibial muscle must, of course, take account of its normal character-

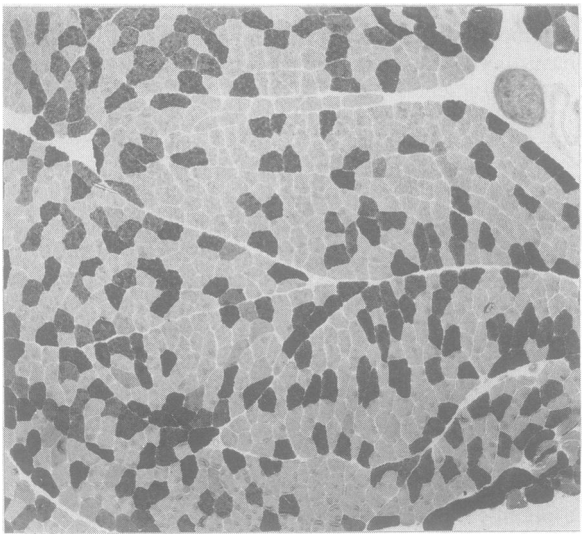

Fig 3 *Normal anterior tibial muscle histology. Note preponderance of pale staining type 1 fibres (Myosin ATPase pH 9·4 × 52).*

istics (fig 3). The type 1 fibre predominance of approximately 70% limits the value of this muscle for detecting subtle changes in fibre type ratios and grouping. Occasional moth-eaten fibres in the normal muscle may initially cause confusion. The superficial situation of the common peroneal nerve renders it vulnerable to local damage, and theoretically this might produce from an anterior tibial biopsy the misleading impression of a generalised neuropathic process. We have not found this to be a problem in practice.

Biopsy of a distal muscle might appear illogical in myopathies, many of which tend to affect proximal muscles preferentially. In many patients with advanced proximal muscle disease, however, extensive replacement with fat and fibrous tissue may make distal muscle sampling preferable. Figure 4 illustrates abnormalities in two muscles from a patient with limb girdle dystrophy. The sample from the vastus lateralis shows features of "end stage" muscle disease, (fig 4a) with fat and fibrous tissue and scattered atrophic muscle fibres. The biopsy taken from the anterior tibial muscle showed abnormalities consistent with a diagnosis of limb girdle dystrophy (fig 4b). Our subsequent experience with the use of conchotome biopsy of the anterior tibial muscle has shown that it is possible to obtain diagnostic information from a wide range of proximal myopathies.

Histological and biochemical techniques available for analysis of relatively small muscle samples have meant that open muscle biopsy is now rarely justified.

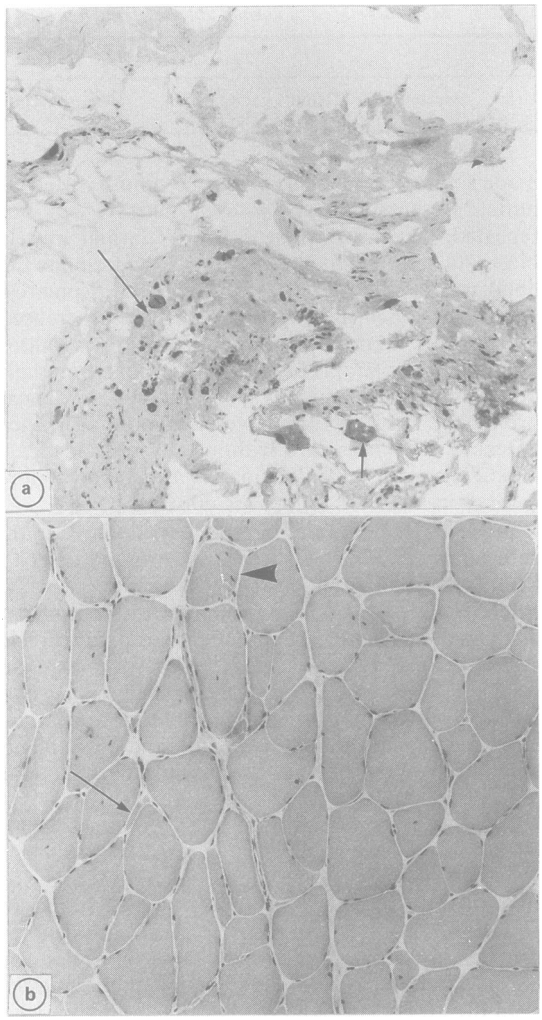

Fig 4(a) *Limb girdle dystrophy: end stage changes in vastus lateralis. Note gross replacement of muscle with fat and fibrous tissue, and only a few scattered atrophic muscle fibres (arrow) (H and E × 130.)* (b) *Limb girdle dystrophy: tibialis anterior muscle showing mild myopathic changes including fibre atrophy (arrow) and hypertrophy, fibre splitting (arrow head), internal nuclei and mild endomysial fibrosis (H and E × 130).*

To secure a diagnosis of vasculitis, open biopsy of muscle and blood vessels may still be required. Large muscle samples are also needed occasionally for analysis of motor end plate function, for certain mitochondrial coupling measurements, and for halothane sensitivity studies in malignant hyperpyrexia. Open biopsy under general anaesthesia is unnecessarily hazardous, and particularly so in children with muscle

disease.[28] For the large majority of clinical situations, percutaneous biopsy gives adequate information whilst being more acceptable ethically and cosmetically. Conchotome biopsy, especially of the anterior tibial muscle, is almost painless and so is particularly well suited to longitudinal studies in patients and normals where repeated biopsies are required. At a time when much is still to be learned about diseases of muscle, and also about muscle wasting in other conditions, the opportunity for repeated access to muscle for review of diagnosis or evaluation of treatment attempts is clearly advantageous.

The Liverpool muscle biopsy needle is a modification of the "Allandale" biopsy needle which can be obtained from Northern Hospital Supplies, Spylaw Street, Colinton, Edinburgh, EH13 0JT, Scotland. They are made in a range of sizes 4·5 mm, 5·0 mm and 6·0 mm diameter. Conchotomes similar to those described here are available from Downs Surgical Ltd, Church Path, Mitcham, Surrey, CR4 3UE, UK. (Tel: 01-648 6291), as Henckel Tilley's Forceps, Cat No. LG-780-45-S, in sizes 4·5–6·5 mm.

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References

1 Edwards RHT, Round JM, Jones DA. Needle biopsy of skeletal muscle: a review of 10 years experience. *Muscle Nerve*. 1982;**6**:676–83.
2 Heckmatt JZ, Moosa A, Hutson C, Maunder-Sewry CA, Dubowitz V. Diagnostic needle muscle biopsy. *Arch. Dis. Child*. 1984;**59**:528–32.
3 Dubowitz V. Muscle biopsy: a practical approach. 2nd Ed. London. Bailliere Tindall, 1985:3–18.
4 Coakley J, Smith PEM, Dietrichson P, Helliwell T, Edwards RHT. Percutaneous muscle biopsy with conchotome. *Clin. Sci*. 1986;**71 (suppl 15)**:23P.
5 Radner S. Knapphalsteknik och iterativ muskelbiopsi. Summenfattning XIX. Rikstamman anordnad av Svenska Lakarsallskapet. 1962:94–5.
6 Henriksson KG. "Semi-open" muscle biopsy technique. A simple out patient procedure. *Acta. Neurol. Scand*. 1979;**59**:317–23.
7 Dietrichson P. Percutan muskelbiopsi med konkotom. Tidsskr. nor. Laegeforen. 1975;**95**:1856–8.
8 Dietrichson P, Mellgren SI, Skre M. Muscle biopsy with the percutaneous conchotome technique. *J. Oslo City Hosp*. 1980;**30**:73–79.
9 Edwards RHT, Coakley J, Stokes M, Wagenmakers AJM, Griffiths RD. A tissue pharmacology approach to treatment trials in muscular dystrophy. *Muscle Nerve*. 1986;**9S**:270.
10 Bulcke JAL, Baert AL. *Clinical and Radiological Aspects of Myopathies*. Springer-Verlag, Berlin, 1982:4.
11 Heckmatt JZ, Leeman S, Dubowitz V. Ultrasound imaging in the diagnosis of muscle disease. *J. Pediatr*. 1982;**101**:656–60.
12 Brown M, Swift TR, Spies SM. Radio-isotope scanning in inflammatory muscle disease. *Neurology*. 1976;**26**: 517–20.
13 Gohil K, Jones DA, Edwards RHT. Fatty acid oxidation from needle biopsy samples of human skeletal muscle. *Clin Sci*. 1984;**66**:173–8.
14 Wagenmakers AJM, Edwards RHT. Tissue effective investigation of mitochondrial metabolism in needle biopsy samples of human skeletal muscle. *Biochem. Soc. Trans*. 1986;**14**:696–7.
15 Helliwell TR, Coakley J, Smith PEM, Edwards RHT. The morphology and morphometry of the normal human tibialis anterior muscle. *Neuropathol. Appl. Neurobiol*. (In Press).
16 Bergstrom J, Hultmann E. The effect of exercise on muscle glycogen and electrolytes in normals. *Scand. J. Clin. Lab. Invest*. 1966;**18**:16.
17 Russell DMcR, Walker PM, Leiter LA, *et al*. Metabolic and structural changes in skeletal muscle during hypocaloric dieting. *Am. J. Clin. Nut*. 1984;**39**:503–13.
18 Novarini A, Borghi L, Curti A, Montanari M, *et al*. Extracellular water, electrolyte and nitrogen balance after post operative parenteral nutrition and intracellular involvement in muscle. *Acta. Clin. Scand*. 1983;**149**:651–6.
19 Guarnieri G, Toigo G, Situlin R, *et al*. Muscle biopsy studies in chronically uraemic patients: Evidence for malnutrition. *Kidney International* 1983;**24(Suppl 16)**: S187–S193.
20 Charriere M, Duchenne GB. Emporte Piece Histologique. *Bull. Acad. Matl. Med*. 1965;**30**:1051–1.
21 Bergstrom J. Muscle electrolytes in man: determined by neutron activation analysis on needle biopsy specimens: a study in normal subjects, kidney patients and patients with chronic diarrhoea. *Scand. J. Clin. Lab. Invest*. 1962;**14(Suppl 68)**:1–110.
22 Edwards RHT. Percutaneous needle biopsy of skeletal muscle in diagnosis and research. *Lancet* 1971;**ii**: 593–6.
23 Cummings WJK, Mahon M. Needle biopsies (letter). *Muscle Nerve* 1984;**7(8)**:679–80.
24 Henriksson-Larsen K. Distribution, total number and size of different types of fibres in male and female skeletal muscles. An enzyme histochemical study of whole muscle cross-sections. Umea University Medical Dissertations, New Series No. 132–ISSN 0346–6612.
25 Henriksson-Larsen K. Distribution, number and size of different types of fibres in whole cross-sections of female m tibialis anterior. An enzyme histochemical study. *Acta. Physiol Scand*. 1985;**123**:229–35.
26 Polgar J, Johnson MA, Weightman D, Appleton D. Data on fibre size in 36 human muscles. An autopsy study. *J. Neurol. Sci*. 1973;**19**:307–18.
27 Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in 36 human muscles. *J. Neurol. Sci*. 1973;**18**:111–29.
28 Willner J, Nakagawa M, Wood D. Drug induced fiber necrosis in Duchenne dystrophy. *Ital. J. Neurol. Sci*. 1984;**Suppl 3**:117–21.